SUSCEPTIBILITY TO PSORIASIS

The present invention concerns diagnostic tests for susceptibility to psoriasis, together with medicaments and methods for treating same.

Psoriasis is a common chronic skin disease with an important genetic component. Association with specific HLA alleles, particularly HLA-B57, CW6 and HLA-DR7, has frequently been reported in scientific journals, and such findings are supported by family based molecular genetic studies showing linkage to chromosome 6p21.3 (Trembath, R. C. *et al.*, 1997, Hum. Mol. Genet., 6(5): 813-820).

It has in fact been suggested that HLA-Cw6 is itself the causative gene for psoriasis. However, the present inventors have found that a non-HLA gene known as the S gene (see for example Zhou, Y. and Chaplin, D. D., 1993, PNAS USA, 90(20): 9470-9474; GenBank accession number L20815; Guerrin, M. *et al.*, 1998, J. Biol. Chem., 273(35): 22640-7 and references therein) which encodes corneodesmosin is strongly associated with susceptibility to psoriasis. Experiments (below) have shown this association to be independent of HLA-C. Other relevant publications include Ahnini, R.T. *et al.*, June 1999, Human Molecular Genetics, 8(6): 1135-1140.

Additional S gene (i.e. corneodesmosin encoding gene) deposits include NM_001264, AJ238467, AJ238466, AJ238465, AJ238464, AJ238463, AJ238462, AJ238461, AI768204, AF030130 and AI582314.

Thus according to the present invention there is provided the S gene for use in a method of treatment or diagnosis of the human or animal body.

As used herein, the term "treatment" is considered in general terms to mean any treatment which is designed to cure, alleviate, remove or lessen the symptoms of, or prevent or reduce the possibility of contracting any disorder or malfunction (i.e. in the case of the present invention, psoriasis) of the human or animal body.

The S gene is polymorphic (and the present invention therefore relates to the gene in its various polymorphic forms) and experiments have shown that different allelic forms of the gene are associated with different levels of susceptibility to psoriasis.

Thus also provided according to the present invention is a diagnostic test method for determining the susceptibility of a patient to psoriasis, comprising the steps of:

i) taking a sample from said patient;

ii) comparing the sequence of the S gene of said patient to that of an S gene causing a predetermined susceptibility to psoriasis; and

iii) correlating the results of comparison step (ii) to determine the susceptibility of said patient to psoriasis.

Comparison step (ii) may comprise determining whether the S gene has a T nucleotide at position 619, a G nucleotide at position 1240 and a C nucleotide at position 1243. As discussed above, the present invention relates to the use of the S gene in its various polymorphic states. However, reference to specific nucleotide positions is made relative to nucleotide positions defined in GenBank accession L20815 (above).

As well as polymorphisms at the above positions, additional diagnostically useful polymorphisms have been found at positions 1236 (a T→G mutation) and 1215

(an A→G mutation). Further polymorphisms (also diagnostically useful) are to be found at positions: 9 (t to c), 66 (a to g), 461-463 (3bp deletion), 614 (a to g), 619 (c to t), 722 (t to c), 767 (g to a or c), 971 (t to c), 1118 (g to a), 1215 (a to g), 1236 (t to g), 1243 (c to t), 1331 (g to a or c) and 1358 (t to c).

Experiments (below) have shown that the polymorphic S gene has an allelic form (referred to herein as allele 5) which is particularly strongly associated with a susceptibility to psoriasis. This means that as well as performing simple tests to compare the entire sequence of a patient's S gene to a reference S gene giving a predetermined susceptibility to psoriasis, the presence or absence of particular allelic forms of the S gene may be used to determine susceptibility to psoriasis, determining whether or not certain nucleotides are present at specific positions within the gene. It is of course possible for a test not to require the sequencing or comparison of the entire gene, instead only determining the sequence at specific positions, for example at positions 619, 1240 an 1243.

Table 2 shows 8 different alleles detectable by the nucleotides at positions 619, 1240 and 1243. Allele 5 has not been previously identified in any studies.

Thus also provided according to the present invention is an isolated S gene having nucleotides T619, T1240 and T1243.

Such a nucleic acid sequence or fragment may be for use in a method of treatment or diagnosis of the human or animal body, particularly for use in determining susceptibility of a patient to psoriasis. Also provided is the use of an S gene having nucleotides T619, T1240 and T1243 or a fragment thereof comprising at least nucleotides 619, 1240 and 1243 thereof in a method of diagnosis of susceptibility to psoriasis. Also provided is the use of the an S gene having nucleotides T619, T1240 and

T1243 or a fragment thereof comprising at least nucleotides 619, 1240 and 1243 thereof in a method of manufacture of a medicament for the treatment of psoriasis.

Such alleles can be readily distinguished by PCR and so the comparison step of a diagnostic test may comprise the step of performing PCR using discriminatory primers for nucleotide substitutions at positions 619, 1240 and 1243 and comparing the results to those obtained with an S gene causing a predetermined susceptibility to psoriasis. The preparation of discriminatory primers is well within the capabilities of a person skilled in the art using the teachings of e.g. PCR (Volume 1): A practical approach. Eds. M.J. McPherson, P. Quirke and G.R. Taylor. Oxford University Press, 1991. Discriminatory primers for nucleotide substitutions at one or more of positions 1236, 1215, 9, 66, 614, 619, 722, 767, 971, 1118, 1215, 1236, 1243, 1331, and 1358 may also be used, as may discriminatory primers for a deletion at positions 461-463 (3bp deletion).

The present invention also provides pairs of PCR primers which may be used to determine the presence or absence of a particular S gene allele and thus to determine susceptibility to psoriasis. Such a pair of PCR primers may have the sequences of any one of the group of SEQ ID NOs: 1 and 5, 1 and 6, 2 and 5, 2 and 6, 1 and 7, 1 and 8, 3 and 5, 3 and 6, 4 and 5, 4 and 6, 3 and 7, 3 and 8, 4 and 7, 4 and 8, 1 and 9, 2 and 9, 1 and 10, 2 and 10, 3 and 9, 4 and 9, 3 and 10, and 4 and 10.

Naturally, other sequencing and comparison technologies may be used as an alternative to PCR. For example, a sample gene may simply be sequenced and the derived sequence then checked for the presence or absence of specific nucleotide substitutions (above). Alternatively, hybridisation studies using fragments of polymorphic forms of the S gene may be performed. Alternatively, techniques such as Western blot analysis, mRNA Northern blot analysis, and RFLP analysis may be used. Similarly, real-time PCR (RT-PCR) techniques such as TaqMan may be used. Such

comparison techniques and others are well known in the art as describe in, for example, Sambrook, J., Fritsch, E.F. and Maniatis, T., "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor Press, New York, 1989.

The various S gene sequences (particularly the coding sequences) for corneodesmosin provide the material for further DNA based primer design to enable both sequencing of individuals for all variants of the S gene for diagnostic purposes and the design of additional methods of genotyping individuals. Methods employed include the design of specific PCR S gene products for restriction enzyme digest analysis (to detect single nucleotide DNA variants) or their detection through the use of hybridisation methodology against fixed labelled probes (oligonucleotide detection methods) and microarray hybridisation for mini-sequencing.

Large scale variants and specific site variants of the S gene can be detected by Southern blot analysis (genomic and cDNA clones as described above) against genomic DNA from patients and individuals under assessment. RNA detection methods include the *in-situ* detection of localisation of the S gene product, the quantification of S gene levels by semi-quantitative PCR and the analysis of protein by Western blotting.

The step of comparing the sequence of the patient's S gene to that of an S gene causing a predetermined susceptibility to psoriasis need not be performed using the actual S gene of the patient. For example, mRNA synthesised from the gene may be used in any comparison. Similarly, the polymorphisms may cause the epitopes displayed by corneodesmosin (which is encoded by the S gene) to differ. The existence and identity of such epitopes may be readily determined using techniques such as epitope mapping and mimotope design (see for example Geysen, H.M *et al.*, 1987, Journal of Immunological Methods, 102: 259-274; Geysen, H.M. *et al.*,1988, J. Mol. Recognit., 1(1):32-41; Jung, G. and Beck-Sickinger, A.G., 1992, Angew. Chem. Int. Ed. Eng., 31: 367-486). Once such epitopes have been identified and isolated, antibodies and antigen

binding fragments thereof may be used in assays to determine the presence (or absence) of the epitopes. Antibodies, antigen binding fragments thereof and their use are well known in the art from e.g. Harlow, E. and Lane, D., "Using Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998

The present invention also provides a diagnostic test kit for determining the susceptibility of a patient to psoriasis, characterised in that it comprises at least one pair of PCR primers according to the present invention.

Also provided according to the present invention is the use of at least one pair of PCR primers according to the present invention in the manufacture of a diagnostic test kit for susceptibility to psoriasis.

Also provided is the use of the S gene in the manufacture of a diagnostic test for psoriasis.

Despite being strongly associated with psoriasis, the S gene is not the sole determining factor - a person having allele 5 of the S gene but no family history of psoriasis may be less likely to suffer from psoriasis than a person having allele 5 of the S gene and having a family history of psoriasis. Thus a diagnostic test based upon the S gene may be supplemented by other information, e.g. about any family history of psoriasis, in order to reach a more conclusive opinion about an individual's susceptibility to psoriasis.

The uses of the S gene are not limited solely to diagnosis - it may be used as the basis for therapies, for example gene therapy (Wolf, J. A. and Crow, J. F. (1994) Gene Therapeutics: Methods and Application of Direct Gene Transfer, Springer Verlag, London). Thus also provided according to the present invention is the use of the S gene in the manufacture of a medicament for the treatment of psoriasis. Also provided is a

method of manufacture of a medicament for the treatment of psoriasis, characterised in the use of the S gene.

Corneodesmosin is metabolised by proteolytic enzymes. Thus therapies may be targeted at modulation of these enzyme, thereby altering the extent of corneodesmosin in skin. Such enzymatic activity could be regulated by for example, medicaments which compete with corneodesmosin as substrate. Such medicaments may be provided topically or systemically. An *in vitro* screening method based on such medicaments may be useful as enabling technology for drug discovery.

Any medicament according to the present invention may additionally comprise other ingredients, for example a pharmaceutically acceptable carrier, diluent or excipient (see for example Remington's Pharmaceutical Sciences and US Pharmacopeia, 1984, Mack Publishing Company, Easton, PA, USA). The exact dosage and treatment regime of any medicament may be readily determined using e.g. dose-response assays.

The invention is not limited solely to the S gene itself - the expression product of the gene (corneodesmosin) can be used therapeutically and diagnostically.

Thus also provided is the use of the corneodesmosin protein or an immunogenic fragment thereof for use in a method of treatment or diagnosis of the human or animal body.

Experiments (below) have shown that the intensity and distribution of staining for corneodesmosin is altered in patients with psoriasis compared with patients with normal skin (Figure 1). These expression studies make use of antibodies to corneodesmosin (Guerrin, M. *et al.*, 1998, *supra*). As used herein, the term "Antibody" also extends to include antigen binding fragments (Harlow, E. and Lane, D., 1988,

Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York; Harlow, E and Lane, D., 1998, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York). Antibodies according to the invention may be of polyclonal of monoclonal origin specific for one or more epitopes.

Thus also provided according to the present invention is a diagnostic test method for psoriasis, comprising the steps of:

i) taking a sample from a patient;

ii) comparing the expression pattern of corneodesmosin protein in said sample to that of a control sample; and

iii) correlating the results of comparison step (ii) to determine the presence of psoriasis in said patient.

Such a test may also be used to determine the susceptibility of a patient to psoriasis, i.e. it may be performed for a patient not actually suffering from psoriasis.

Therefore also provided by the present invention is a diagnosis test method for diagnosing the susceptibility of a patient to psoriasis, comprising the steps of:

i) taking a sample from said patient;

ii) comparing the expression pattern of corneodesmosin protein in said sample to that of a control sample; and

iii) correlating the results of comparison step (ii) to determine the susceptibility to psoriasis in said patient.

A control sample could for example be a sample from a patient either suffering or not suffering from psoriasis. Where a diagnostic test is for susceptibility to psoriasis, a control sample could be a sample from a patient having a predetermined susceptibility to psoriasis.

It is also possible to generate antibodies specific for the corneodesmosin protein expressed by S gene allele 5, which is strongly associated with susceptibility to psoriasis, or other associated S gene alleles. Such antibodies may be specific against epitopes displayed by particular S gene alleles, as discussed above, and may be used in the various aspects of the present invention.

Also provided according to the present invention is the use of antibody specific to corneodesmosin in the manufacture of a diagnostic test kit for the presence of, or susceptibility to, psoriasis.

The present invention also provides a method of manufacture of a medicament for the treatment of psoriasis, characterised in the use of corneodesmosin or antibody specific against same.

The invention will be further apparent from the following description which shows by way of example only tests for susceptibility to psoriasis.

Of the Figures:

Figure 1 shows corneodesmosin cell surface staining in normal patients (Figure 1a) and in psoriasis sufferers (Figure 1b). Staining was with anti-corneodesmosin antibodies. Figure 1a shows (10) a thin discrete layer of dense staining where corneodesmosin is to be found in normal patients. Figure 1b (psoriasis sufferer) shows (20) shows thick bands of staining for corneodesmosin.

Experiments

The polymorphic S gene lies 160 kb telomeric of HLA-C and its expression is restricted to differentiating epidermal keratinocytes. Studies of parent-offspring trios show significant evidence for linkage and disease association with allele 5 (see Table 2) of the S gene. Linkage studies show S gene involvement in psoriasis being independent of HLA-C. Corneodesmosin expression studies in patients with psoriasis show an altered expression pattern when compared to those of non-psoriasis patients.

Parent-Offspring Study

A total of 152 parent-offspring trios from 99 independent psoriatic Caucasoid kindreds were genotyped using a PCR approach that identified the S gene polymorphisms inherited in cis. To minimise the effect of population stratification, the number of occasions an allele was transmitted to an affected individual was compared to the control frequency of non-transmission (transmission disequilibrium test (TDT) (Lazzeroni, L. C. and Lange, K., 1998, Hum. Hered., 48(2): 67-81)). Significant evidence for linkage and disease association was observed for the S gene defined as allele 5 ($p<0.000003$) (Table 3) in contrast to only weak support for individual informative polymorphisms (1243 (c) $p<0.0005$).

In order to assay for the presence of S gene alleles having substitutions at positions 619, 1240 and 1243, PCR amplification using primers specific for substitutions at positions 619, 1240 and 1243 was used.

Sense primers

A synonymous polymorphism is present at position 614 (an A→G substitution) which could interfere with primer binding and so two versions of each primer were produced and used in equimolar amounts. The first two primers (SEQ ID NOs: 1 and 2) were for a "normal" (GenBank accession no. L20815) sequence corresponding to nucleotides 600-

619, and are referred to as the 619 C primers. The second two primers (SEQ ID NOs: 3 and 4) were for the C→T substitution at position 619, and are referred to as the 619 T primers.

Antisense primers

Two sets of primers were made - one for the substitution at position 1240 and one for the substitution at position 1243.

The set of primers for the 1240 substitution had to be unaffected by any substitutions at position 1243, and this necessitated the synthesis of two pairs of primers. The first pair (referred to as the 1240 G primers) had SEQ ID NOs: 5 and 6 corresponding to the normal G nucleotide at position 1240. The second pair (referred to as the 1240 T primers) had SEQ ID NOs: 7 and 8 corresponding to the G→T substitution at position 1240.

The set of primers for the 1243 substitution comprised two primers SEQ ID NO: 9 (referred to as the 1243 C primer), corresponding to the normal nucleotide C at position 1243, and SEQ ID NO: 10 (referred to as the 1243 T primer), corresponding to the C→T substitution at position 1243.

PCR was performed using either one of the pairs of sense primers (i.e. the 619 C primers or 619 T primers) and either one of the 1240 primer pairs (i.e. the 1240 G primers or 1240 T primers) or either one of the 1243 primers (i.e. the 1243 C primer or the 1243 T primer). This gave 8 different combinations of PCR primers (Table 1). With each sample to be analysed, each set of PCR primers was used and the results of PCR amplification (i.e. successful or unsuccessful) analysed in order to determine the nucleotides at each of positions 619, 1240 and 1243. This allowed the identification of 8 alleles of the S gene (Table 2). Results of this study allowed the correlation of the occurrence of psoriasis with particular alleles of the S gene, allowing the identification of allele 5 as being strongly associated with psoriasis.

Final result for the parent-offspring study are given in Table 3.

Linkage study

Extensive linkage disequilibrium is observed between loci within the MHC and confounds genetic attempts to map genes within this region. Chromosome 6p21.3 haplotypes were constructed for a 500kb region for 8 genetic markers spanning the interval from "D6S276" to "D6S291" which includes HLA-C and S gene. Amongst informative parents (heterozygotes) 38% of S gene allele 5 transmissions were independent of the disease-associated HLA-Cw6 bearing haplotype. Hence, these novel data provide genetic support for the S gene in psoriasis susceptibility which may be independent of HLA-C. Additionally, these studies define a specific disease related S gene allele.

Corneodesmosin expression in psoriasis

A number of monoclonal antibodies to corneodesmosin have been produced (Guerrin, M. *et al.*, 1998, *supra*) and these were used to localise this protein in human skin.

5mm cryostat sections of psoriasis and normal skin were immunostained with two monoclonal antibodies, G36-19 and F28-27, using a peroxidase anti-peroxidase technique as follows. Sections were air dried, fixed in acetone and non-specific antibody binding blocked by pre-treating the tissue with normal swine serum. Corneodesmosin specific antibodies were applied at a dilution of 1/100 for 60 minutes at room temperature. Peroxidase conjugated rabbit anti-mouse immunoglobulin and mouse peroxidase anti-peroxidase complex were sequentially applied to the tissue each for 30 minutes at room temperature. Bound antibody was then visualised by incubating the tissue with a substrate solution containing hydrogen peroxide and 3,3' diaminobenzidine and lightly counterstaining the sections with Mayers haemalum.

In normal skin corneodesmosin cell surface staining was present in the compressed cells of the stratum granulosum, throughout the full thickness of the stratum corneum and in the basal layer (Figure 1a). In psoriasis, basal cell expression was attenuated and in the upper layers of the epidermis expression was perinuclear and absent from the cell surface (Figure 1b). Expression within the parakeratotic stratum corneum was also attenuated. Thus both the intensity and distribution of staining for corneodesmosin is altered in psoriasis compared with normal skin.

In humans corneodesmosin is a late differentiation component of desmosomes in the granular layer and corneodesmosomes in the stratum corneum. Corneodesmosin is thought to function in cell cohesion in the stratum corneum with degradation of the protein leading to epidermal desquamation. Desquamation is a fundamental process in the pathogenesis of psoriasis. The altered expression in psoriasis is consistent with a primary role in disease pathogenesis.

TaqMan assay

An A→G mutation at position 1215 and a T→G mutation at position 1236 have also been found to be diagnostically useful for determining a patient's susceptibility to psoriasis. In order to screen patient samples for these two mutations, the following probes and primers (supplied by Perkin Elmer and EuroGenTec) were used in a TaqMan assays:

S gene SNP1215A probe CGA GTC CCC AGC AGT TCT AGC ATT TC (SEQ ID NO: 21)

VIC (probe label)

S gene SNP1215G probe CGA GTC CCC AGC GGT TCT AGC A (SEQ ID NO: 22)

FAM(probe label)

SgeneSNP1215 forward primer ACC CTG CTC TCC CTC CAG TT (SEQ ID NO: 23)

SgeneSNP1215 reverse primer ACT GCC GCA GGG ATG GTA (SEQ ID NO: 24)

SgeneSNP1236T probe TCT AGC ATT TCC AGC AGC TCC GGT T (SEQ ID NO: 25)

[5']TET (probe label)

[3']TAMRA(probe label)

SgeneSNP1236G probe CAT TTC CAG CAG CGC CGG TT [5'}6-FAM (SEQ ID NO: 26)

SgeneSNP1236 forward primer ACC CTG CTC TCC CTC CAG TT (SEQ ID NO: 27)

SgeneSNP1236 reverse primer ACT GCC GCA GGG ATG GTA (SEQ ID NO: 28)

The TaqMan assay is a real-time PCR (RT-PCR) assay and work by mixing a pair of probes (for example the SgeneSNP1236T probe and the SgeneSNP1236G probe) with a sample from a patient. The probes use fluorophore-quencher combinations and are disabled from extension at the 3' end. During PCR (using for example the SgeneSNP1236 forward and reverse primers) the probes are allowed to hybridize to their complementary single stranded DNA sequences within the PCR target. If a specific target is not present then hybridisation will not occur. When amplification occurs, the hybridised probes are degraded by the 5'→3' exonuclease activity of the *Taq* DNA polymerase. This degradation of the hybridised probe causes the separation of its fluorophore and quencher moieties and results in detectable fluorescence from the fluorophore. Thus fluorescence is indicative of the presence of a sequence to which a probe was specific. A plurality of probes having different fluorophores having different excitation and emission spectra may therefore be used, allowing the detection of the presence of one or more specific sequences from a range covered by the set of probes.

In the assay, aliquots of 1 microlitre of genomic DNA (40ng/microlitre) are plated into 96 well optical plates, 24 microlitres of a TaqMan (RTM) Universal mastermix containing standard PCR reagents, one of the two pairs of forward and reverse primers at 300-900 nM and both probes for the sequence amplified by the primers at 100-200 nM is added to each well. The wells are sealed with optical caps and the plate loaded into a 7700 Sequence Detector System (Perkin Elmer ABI) - the TaqMan machine. Thermal cycling is performed at 40 cycles of 50 °C for 2 minutes, 95 °C for 10 seconds, 95 °C for 15 seconds and 62 °C for 1 minute, after 1 hour 56 minutes amplification is complete and the fluorescent signal from each well is read automatically.

DNAs are grouped according to genotype into allele 1 or allele 2 homozygotes or allele 1/2 heterozygotes. Genotypes called automatically can be checked against internal control DNAs, previously genotyped by DNA sequencing, which are included in each plate. Further confirmation of genotyping can be obtained if required, by reading real time plots of fluorescence intensity collected separately for each well during the PCR amplification period.

Diagnostic Protein Detection

Example 1. Immunofluorescent detection (i.e. direct detection) of corneodesmosin in its various polymorphic forms is performed according to the following steps:

i) monoclonal antibodies are prepared based on wild type and mutated corneodesmosin, predicted from S gene sequence and are fluoresceinated (Harlow, E. and Lane, D., "Using Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998);

ii) tissue sections of skin biopsies are cut with microtome and placed on glass slides;

iii) fluoresceinated primary antibody is added to tissue section;

iv) after incubation, antibody is washed off;

v) glass coverslip is applied to section/slide;

vi) slide is viewed through optical microscope using ultraviolet light. Antibodies to wild type corneodesmosin show altered expression, and antibodies to mutated form show expression only in psoriasis.

Example 2. Enzyme linked immunosorbent assay (ELISA) for corneodesmosin in its various polymorphic forms is performed according to the following steps:

i) a sample of either peripheral blood (serum/plasma) or aqueous extracts of stratum corneum (scale) of lesional skin is taken from a patient;

ii) antibodies as in Example 1 (above) are immobilised in wells in 36 well plates;

iii) substrate as in Example 1 (above) is added to the wells;

iv) after incubation and washing, corneodesmosin adheres to immobilised antibody;

v) colorimetric substrate linked-anticorneodesmosin antibody is added to the wells;

vi) enzyme substrate is added to produce a coloured product in the presence of corneodesmosin, and the results are read by automated colorimetry.

Additional S gene sequence variants

Using the genomic corneodesmosin sequence (accession number AC006163) additional sequence variants in the S gene coding sequence were identified. Primer pairs designed from the AC006163 genomic sequence were as follows:

Exon 1: forward - SEQ ID NO: 11
reverse - SEQ ID NO: 12

Exon 2:

part 1 forward - SEQ ID NO: 13

	reverse - SEQ ID NO: 14
part 2	forward - SEQ ID NO: 15
	reverse - SEQ ID NO: 16
part 3	forward - SEQ ID NO: 17
	reverse - SEQ ID NO: 18
part 4	forward - SEQ ID NO: 19
	reverse - SEQ ID NO: 20

These primers allowed the detection of the following mutations: 9 (t to c), 66 (a to g), 461-463 (3bp deletion), 614 (a to g), 619 (c to t), 722 (t to c), 767 (g to a or c), 971 (t to c), 1118 (g to a), 1215 (a to g), 1236 (t to g), 1243 (c to t), 1331 (g to a or c) and 1358 (t to c).

Table 1

Primer mixes

i	619 C + 1240 G
ii	619 C + 1240 T
iii	619 T + 1240 G
iv	619 T + 1240 T
v	619 C + 1243 C
vi	619 C + 1243 T
vii	619 T + 1243 C
viii	619 T + 1243 T

Table 2

Alleles	+ ve PCR reactions	Nucleotide position		
		619	1240	1243
001	i + v	C	G	C
002	i + vi	C	G	T
003	ii + v	C	T	C
004	ii + vi	C	T	T
005	iii + vii	T	G	C
006	iii + viii	T	G	T
007	vi + vii	T	T	C
008	vi + viii	T	T	T

Table 3

TDT for S gene polymorphisms in psoriasis family trios

S gene Polymorphism	transmitted	not transmitted	TDT-2in(L) difference	p-value
619 (t)	48	33	2.37	0.06
1243 (c)	68	33	11.63	0.0003
Allele 5	81	32	20.66	0.0000028

Allele 5 (frequency=0.43) of the S gene is defined by intragenic bi-allelic polymorphisms at 619 (t: freq=0.81, heterogeneity=0.3), 1240 (g: freq=0.98, het=0.04) and 1243 (c: freq=0.58, het=0.46) of cDNA sequence.